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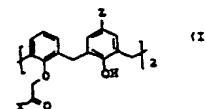
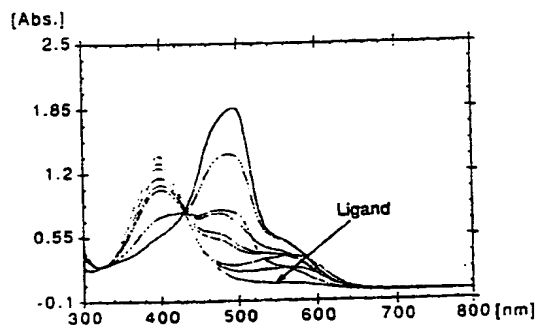
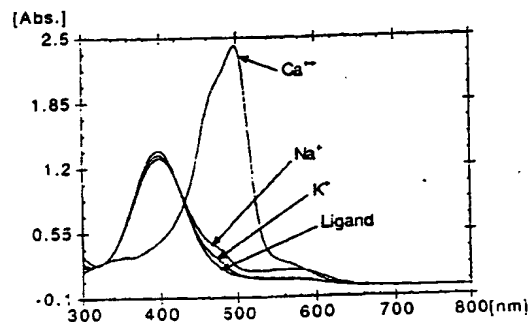
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁵ : C07C 245/08, 255/40, G01N 33/84</p>	<p>A1</p>	<p>(11) International Publication Number: WO 95/00473 (43) International Publication Date: 5 January 1995 (05.01.95)</p>
<p>(21) International Application Number: PCT/DK94/00254 (22) International Filing Date: 22 June 1994 (22.06.94) (30) Priority Data: 0742/93 23 June 1993 (23.06.93) DK (71) Applicant (for all designated States except US): RADIOMETER MEDICAL A/S [DK/DK]; Emdrupvej 72, DK-2400 Copenhagen NV (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): BYRNARD, Allan, Milton [DK/DK]; 5th floor, Hollænderdybet 33, DK-2300 Copenhagen S (DK). UNGARO, Rocco [IT/IT]; Via Ermes Benaglia, 14, I-43040 Vicofertile (IT). POCHINI, Andrea [IT/IT]; Borgo P. Cocconi, 11, I-43100 Parma (IT). (74) Common Representative: RADIOMETER MEDICAL A/S; Patent Department, Emdrupvej 72, DK-2400 Copenhagen NV (DK).</p>		<p>(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.</p>

(54) Title: CHEMICAL COMPOUND

(57) Abstract

A novel calix[4]arene compound, application of the compounds as an active component in a calcium sensitive sensor, and a calcium sensitive sensor containing the compound. The calix[4]arene compound has general formula (I). The sensor is not very sensitive to sodium and potassium ions.



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CHEMICAL COMPOUND

This invention relates to a novel chemical compound,
the application of the compound as an active component
5 in a calcium sensitive sensor, and a calcium sensitive
sensor containing the compound. More particularly, the
compound is a derivative of calix[4]arene.

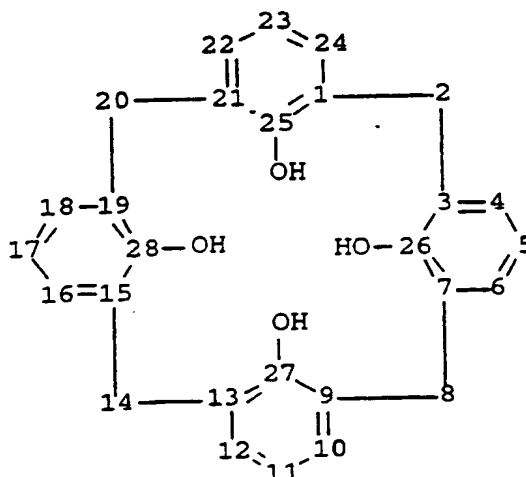
Calixarenes comprise a class of cyclic compounds pre-
10 pared from p-alkylphenols and formaldehyde in the pres-
ence of a catalytic amount of a base. Calixarenes are
disclosed in Gutsche CD. Calixarenes. Acc Chem Res
1983; 16: 161-70. The synthesis procedures for ca-
lix[4]arene, calix[6]arene and calix[8]arene suggested
15 by Gutsche CD are disclosed in Organic Synthesis 1989;
68: 234-46.

Calix[4]arene is usually represented as follows:

20

25

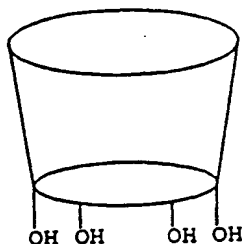
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or

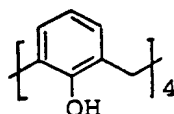
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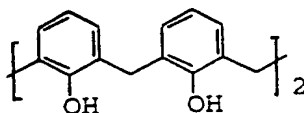
or

10



or

15



20 and the systematic IUPAC term for calix[4]arene is:

pentacyclo[19,3,1,1^{3,7},1^{9,13},1^{15,19}]-octacosa-1(25),3,5,7
(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,
28-tetrol.

25

The ion binding properties of calixarenes have recently
been recognized, see e.g. Arduini A et al. The prepara-
tion of a new lipophilic sodium selective ether ester
ligand derived from p-t-butylcalix[4]arene. Tetrahedron
1986; 42: 2089-100 and Arduini A et al. p-t-butyl-
calix[4]arene tetra-acetamide: a new strong receptor
for alkali cations. J Incl Phenom 1988; 6: 119-34. The
use of calixarenes in ion selective electrodes is dis-
closed in the following scientific papers and patents:

35

Kimura K et al. Lipophilic calix[4]arenes ester and amide derivatives as neutral carriers for sodium ion-selective electrodes. Chem Lett 1988; 615-16;

- 5 Cadogan A et al. Sodium-selective polymeric membrane electrodes based on calix[4]arene ionophores. Analyst 1989; 114: 1551-54;

- 10 Cunningham K et al. Sodium-selective poly(vinyl chloride) membrane ion-selective electrode based on a novel calix[4]arene ionophore. Analytical Proceedings 1991; 28: 294-96;

- 15 Harris SJ et al. European Patent Application No. EP 0490631. Ion selective electrodes; and

Shono et al. Japanese Patent Publication 1-250750 (1989). Sodium ion-selective membrane electrode.

- 20 To particular applications optical ion selective sensors are preferred over ion selective electrodes. Optical sensors based on calixarenes and/or the ion binding properties of calixarenes are disclosed in the following scientific papers:

- 25 Deng G et al. Light-responsive metal encapsulation in calix[4]arene. Chem Lett 1992; 1287-90;

- 30 Shimizu et al. Chromogenic calix[4]arene. Chem Lett 1991; 2147-50;

Kubo Y et al. New chromoionophores based on indoaniline dyes containing calix[4]arene. Tetrahedron Lett 1991; 32: 7419-20;

Jin T et al. A fluorescent calix[4]arene as an intramolecular excimer-forming Na^+ sensor in nonaqueous solution. *J Chem Soc Chem Commun* 1992: 499-501;

- 5 McCarrick M et al. Novel chromogenic ligands for lithium and sodium based on calix[4]arene tetraesters. *J Chem Soc Chem Commun* 1992: 1287-89;

- 10 King AM et al. A highly selective chromoionophore for potassium based upon a bridged calix[4]arene. *J Chem Soc Chem Commun* 1992: 582-84; and

- 15 Kubo Y et al. Synthesis of a 1,3 bis(indoaniline)-derived calix[4]arene as an optical sensor for calcium ion. *J Chem Soc Chem Commun* 1993: 305-307.

The only published work so far dealing with a calcium sensitive calixarene based optical sensor is thus Kubo's above-mentioned 1993 paper.

20

From the data disclosed by Kubo it is obvious that the selectivity for calcium ions towards potassium and sodium ions is inadequate in case the optical sensor is to be used for measurement of physiological fluids such as blood, plasma, serum, etc.

25

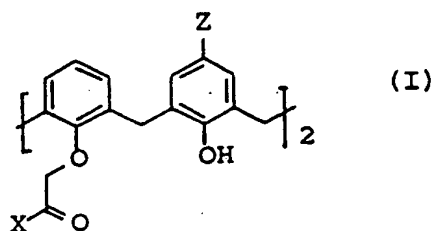
Further, Kubo's calixarene compound cannot stand sterilization. The compound will be destroyed when subjected to radiation sterilization or ETO sterilization. Due to the fact that in some physiological applications, particularly the invasive application, it is essential to use sterilized sensors, sensors based on the calixarene compounds of Kubo are unsuitable for these applications.

30
35

It is an object of the present invention to provide a novel calix[4]arene compound having improved selectivity properties for calcium ions and being more stable during sterilization than present calcium sensitive calix[4]arene derivatives.

The object is accomplished by the calix[4]arene compound according to the invention, said compound being characterized by the general formula

10



15

wherein

20

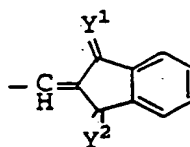
X is -OH, -OR¹, -NR², or morpholino,

whereby R¹ is straight chain or branched alkyl of 1-22 C-atoms and R² is straight chain or branched alkyl of 1-12 C-atoms, and

25

Z is -N=N-Ar, -CH=CH-Ar, -CH=CZ¹Z² or

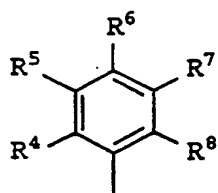
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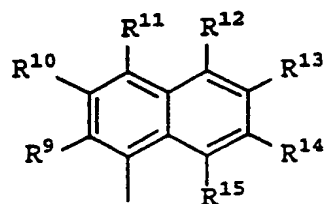
whereby either of Z^1 and Z^2 are selected from -H, -NO₂, -CN, -CF₃, -SOR³, -SO₂R³, -SO₂OR³, -SO₂NHR³, -SO₃H, -COOR³, -COONR³, -COONHR³, -COOH, -CHO, -COR³, -F, -Cl and -Br, R³ is straight chain or branched alkyl of 1-4 C-atoms and both of Z^1 and Z^2 are not -H;

either of Y^1 and Y^2 are selected from =O, =N-CN and =C(CN)₂; and

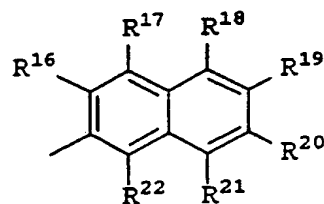
Ar is



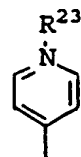
(Substituted phenyl),



(Substituted 1-naphtyl),



(Substituted 2-naphtyl) or

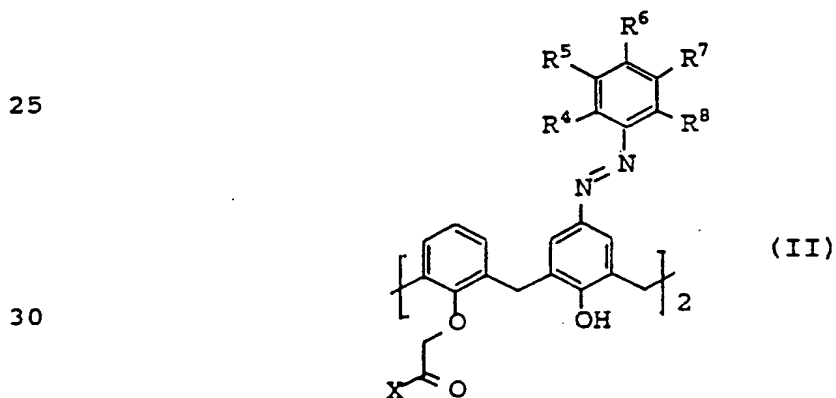


(4-Pyridylum),

and R^4, R^5, \dots, R^{22} are each selected from $-H$, NO_2 ,
 $-CN$, $-CF_3$, $-F$, $-Cl$, $-Br$, $-SOR^{24}$, SO_2R^{24} ,
 $-SO_2CH_2CH_2OR^{25}$, $-SO_2OR^{24}$, $-SO_2NHR^{24}$, $-SO_3H$, $-COOR^{24}$,
 $-CONR^{24}_2$, $-CONHR^{24}$, $-COOH$, $-CHO$ and $-COR^{24}$, wherein
 5 R^{24} is straight chain or branched alkyl of 1-4 C-
 atoms, and R^{25} is $-H$, $-SO_3H$, $-SO_3Li$, $-SO_3Na$ or $-SO_3K$,

with the proviso that when Z is $-CH=CHAR$ and Ar is
 phenyl, at least one of the substituents
 10 R^4, R^5, \dots, R^8 of the phenyl group must be different
 from H , and when Z is $-CH=CHAR$ and Ar is 1-naph-
 thyl, at least one of the substituents $R^9, R^{10}, \dots, R^{15}$
 of the 1-naphthyl group must be different from H ,
 and when Z is $-CH=CHAR$ and Ar is 2-naphthyl, at
 15 least one of the substituents $R^{16}, R^{17}, \dots, R^{22}$ of the
 2-naphthyl group must be different from H .

Preferred compounds are compounds of the type (I)
 wherein Ar is a phenyl group having at least one sulph-
 20 oxylate substituent, particularly compounds of the
 general formula



wherein R^4, R^5, \dots, R^8 are each selected from -H and $-\text{SO}_2\text{CH}_2\text{CH}_2\text{OR}^{25}$; R^4, R^5, \dots, R^8 not all being H, R^{25} has the meaning stated above, and X is -OH or $-\text{OR}^1$, whereby R^1 has the meaning stated above.

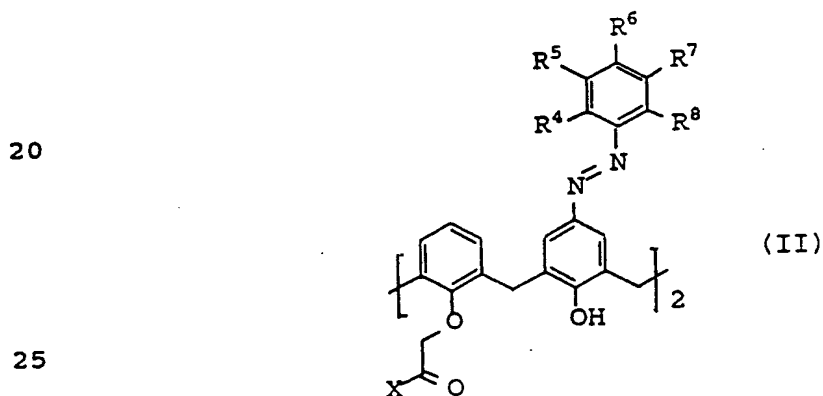
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Particularly preferred compounds are compounds of the type (II) wherein R^{25} is $-\text{SO}_3\text{H}$, $-\text{SO}_3\text{Li}$, $-\text{SO}_3\text{Na}$ or $-\text{SO}_3\text{K}$, as said compounds are suitable for being bound covalently to polymers with available -OH groups, e.g. cel-

10

Other preferred compounds are compounds of the type (I) wherein Ar is a phenyl group having a least one substituent of the type $-\text{NO}_2$, $-\text{CN}$, $-\text{Cl}$, particularly com-

15



wherein R^4, R^5, \dots, R^8 are each selected from -H, $-\text{NO}_2$, $-\text{CN}$ and $-\text{Cl}$; R^4, R^5, \dots, R^8 not all being H, and X is -OH or $-\text{OR}^1$, whereby R^1 has the meaning stated above.

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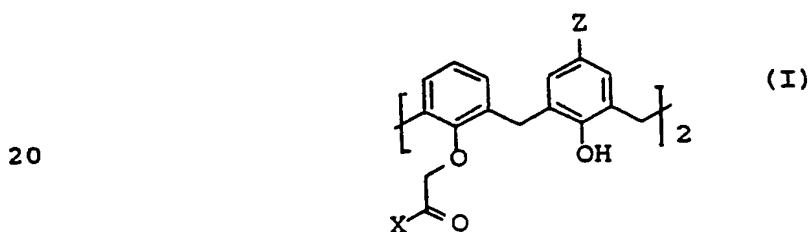
Particularly preferred compounds are compounds of the type (II) where in at least one of the substituents R^4, R^5, \dots, R^8 of the phenyl group is $-\text{NO}_2$ and the others

35

are -H, particularly 4-nitrophenyl and 2,4-dinitrophenyl.

The invention also relates to application of any of the compounds mentioned above of the general formulae (I) and (II) and the particularly preferred compounds mentioned above as an active component in a calcium sensitive sensor.

The invention also relates to a calcium sensitive sensor having a calcium sensitive area containing an immobilized calcium sensitive active component, said calcium sensitive sensor being characterized in that the calcium sensitive active component is a compound of the general formula



25 wherein

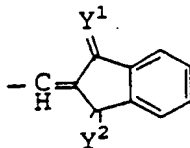
X is -OH, -OR¹, -NR², or morpholino,

whereby R¹ is straight chain or branched alkyl of 1-22 C-atoms and R² is straight chain or branched alkyl of 1-12 C-atoms, and

Z is -N=N-Ar, -CH=CH-Ar, -CH=CZ¹Z² or

35

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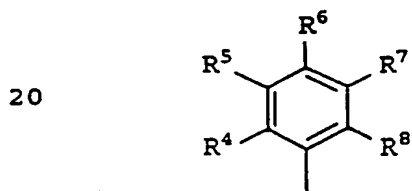


5

whereby either of Z^1 and Z^2 are selected from $-H$,
 $-NO_2$, $-CN$, $-CF_3$, $-SOR^3$, $-SO_2R^3$, $-SO_2OR^3$, $-SO_2NHR^3$,
 $-SO_3H$, $-COOR^3$, $-COONR^3$, $-COONHR^3$, $-COOH$, $-CHO$,
 $-COR^3$, $-F$, $-Cl$ and $-Br$, R^3 is straight chain or
 10 branched alkyl of 1-4 C-atoms and both of Z^1 and Z^2
 are not $-H$;

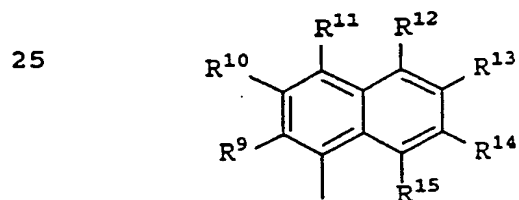
either of Y^1 and Y^2 are selected from $=O$, $=N-CN$ and
 15 $=C(CN)_2$; and

Ar is



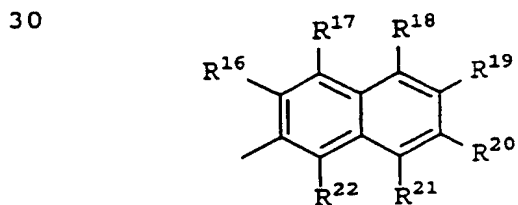
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(Substituted phenyl),



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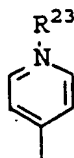
(Substituted 1-naphthyl),



30

(Substituted 2-naphthyl) or

35



(4-Pyridylum),

5

and R^4, R^5, \dots, R^{22} are each selected from $-H$, NO_2 ,
 $-CN$, $-CF_3$, $-F$, $-Cl$, $-Br$, $-SOR^{24}$, SO_2R^{24} ,
 $-SO_2CH_2CH_2OR^{25}$, $-SO_2OR^{24}$, $-SO_2NHR^{24}$, $-SO_3H$, $-COOR^{24}$,
 10 $-CONR^{24}_2$, $-CONHR^{24}$, $-COOH$, $-CHO$ and $-COR^{24}$, wherein
 R^{24} is straight chain or branched alkyl of 1-4 C-
 atoms, and R^{25} is $-H$, $-SO_3H$, $-SO_3Li$, $-SO_3Na$ or $-SO_3K$,

with the proviso that when Z is $-CH=CHAr$ and Ar is
 15 phenyl, at least one of the substituents
 R^4, R^5, \dots, R^8 of the phenyl group must be different
 from H , and when Z is $-CH=CHAr$ and Ar is 1-naph-
 thyl, at least one of the substituents $R^9, R^{10}, \dots, R^{15}$
 of the 1-naphtyl group must be different from H ,
 20 and when Z is $-CH=CHAr$ and Ar is 2-naphtyl, at
 least one of the substituents $R^{16}, R^{17}, \dots, R^{22}$ of the
 2-naphtyl group must be different from H .

Particularly preferred calcium sensitive sensors con-
 25 tain as an active component any of the preferred com-
 pounds mentioned above.

The calcium sensitive area must be located such that it
 will contact the sample when using the sensor. Thus,
 30 the calcium sensitive area must be located on the sur-
 face of the sensor facing the sample.

For practical applications the calcium sensitive active
 component will most often be immobilized in a polymeric
 35 membrane.

To ensure good contact between a sample whose calcium content is to be determined and the calcium sensitive active component, the polymeric membrane is preferably a hydrophilic polymeric membrane, especially a membrane
5 provided from one of the following compounds: celluloseacetate, cellophane, cuprophane, polyvinylacetate, polyhydroxyethylmethacrylate (poly-HEMA) or another hydrogel.

10 In another preferred embodiment the calcium sensitive area comprises a calcium permeable membrane, and the calcium sensitive active component is located in a compartment of the sensor adjacent the membrane.

15 The sensor may be constituted by a so-called dipping sensor, usually rod-shaped, the calcium sensitive area of which is located at one end of the sensor on the surface of the sensor facing the surroundings. The sensor may also constitute a part of a measuring cu-
20 vette designed for containing a sample. In the latter case, the sensor will most often constitute a measuring cuvette wall part. The measuring cuvette may be designed for disposable use or may be provided as an integral component of an analyzer for the determination
25 of the calcium content in samples, preferably physiological samples.

The invention will be further described by the following experiments and in connection with the drawing
30 where:

Fig. 1 shows absorption spectra for a preferred calix[4]arene compound according to the invention in the absence of metal ions and with the addition of potassium, sodium and calcium ions;
35

Fig. 2 shows absorption spectra for the same calix-[4]arene compound having a varying content of calcium ions.

5 The spectra shown in Fig. 1 and Fig. 2 are recorded on an absorption spectrophotometer of the type Kontron UVIKON-860. Both figures show a spectrum of a solution of $5.5 \cdot 10^{-3}$ mol/L of compound (4) described below, i.e. 5,17-bis(4-nitrophenyldiazo)-26,28-dihydroxy-25,27-
10 bis(ethoxycarbonylmethoxy)calix[4]arene in 96% ethanol/tetrahydrofuran (2:1 v/v). In the figure the compound (4) is designated "ligand". In Fig. 1 is also shown spectra of the same solution to which is added $6.67 \cdot 10^{-3}$ mol/L of sodium, potassium and calcium per-
15 chlorate, respectively. It is seen that addition of calcium ions displaces the absorption peak by 100 nm from 397 nm to 497 nm, whereas addition of sodium and potassium ions only results in a negligible change of the absorption spectrum.

20 Fig. 2 shows, apart from the spectrum of the pure ligand solution, spectra of the same solution to which is added varying quantities of calcium ions corresponding to calcium concentrations of $3.33 \cdot 10^{-4}$; $6.67 \cdot 10^{-4}$;
25 $3.33 \cdot 10^{-5}$; $6.67 \cdot 10^{-5}$; $3.33 \cdot 10^{-4}$; $6.67 \cdot 10^{-4}$; $3.33 \cdot 10^{-3}$ and $6.67 \cdot 10^{-3}$ mmol/L. As seen, the absorbance varies clearly with varying calcium concentrations at the absorption peak. Thus, it will be possible to establish a mathematical model or a standard curve from which the con-
30 tent of calcium ions in an unknown sample may be determined.

Experimental

Qualitative determination of calcium with a glass sensor

2 mg of compound (6) described below, i.e.

5 5,17-bis-(2,4-dinitrophenyldiazo)-26,28-dihydroxy-
25,27-bis(hydroxycarbonylmethoxy)calix[4]arene, is
added to a mixture of 2.3 mL water, 2.5 mL methanol and
2.5 mL tetramethoxysilane. 5 drops of 0.1 M KOH is
added with stirring. The reaction mixture was left for
10 4 days in order to gel (formation of a glass) for 4
days in a beaker (6 cm diameter) and was then vacuum-
dried for 2 hours at 40°C.

The glass formed was then washed thoroughly with dilut-
15 ed HCl and distilled water.

A piece of the glass was brought in contact with 0.1 M
aqueous solutions of sodium chloride, potassium chlo-
ride and calcium chloride. In the solution of calcium
20 chloride the colour of the glass changed. In the solu-
tions of sodium chloride and potassium chloride there
was no visually detectable change of colour.

Preparation of calix[4]arene compounds and intermedi- 25 ates therefor

The compounds prepared are characterized by data for
melting point, NMR, IR, by molecular weight determined
by mass-spectrophotometry and by the result of a funda-
mental analysis.

30

The melting points were measured by means of a digital
thermometer.

NMR data were recorded on the following instruments:

35 Bruker AM-100, Bruker AM-250 and Varian Unity 400 spec-
trometer.

IR spectra were recorded using KBr technique on a Perkin Elmer FT-IR 1760X spectrometer.

UV/Vis spectra were measured at room temperature on a Kontron UVIKON-860 and a Perkin Elmer Lambda-9.

- 5 Some of the microanalyses differ more than one would normally accept. This is due to incomplete removal of small neutrals molecules included in the lipophilic cavity of calix[4]arene, e.g. solvent molecules like: CH_2Cl_2 , EtOAc, toluene, etc.

10

25,27-dihydroxy-26,28-bis(ethoxycarbonylmethoxy)calix[4]arene (1)

- 1 g (2.4 mmol) calix[4]arene, 0.33 g (2.4 mmol) anhydrous K_2CO_3 and 0.79 g (0.53 mL; 4.7 mmol) ethylbromacetate are mixed together in a 100 mL roundbottom flask, and 50 mL dry CH_3CN is added. The reaction mixture is heated to reflux for 18 h. The solvent is evaporated and the residue is extracted with CH_2Cl_2 /5% HCl. The organic layer is separated and dried with MgSO_4 .
15 After evaporating the solvent the residue is triturated with MeOH and heated to boiling and cooled to 5°C, then filtered and washed with MeOH.

Yield: 1.0 g (71%).

Melting point [176-177]°C

- 25 ^{13}C NMR (CDCl_3) 14.19, 31.54, 61.42, 72.40, 119.16, 125.82, 128.22, 128.54, 129.20, 133.18, 152.40, 153.04, 169.90

- ^1H NMR (CDCl_3) δ (100 MHz) 1.35(t, 6H, $J=7.20$ Hz), 3.39 (d, 4H, $J=13$ Hz), 4.38(q, 4H, $J=7.20$ Hz), 4.48(d, 4H, $J=13$ Hz), 4.72(s, 4H), 6.71-7.42(m, 12H), 7.62(s, 2H)
30

M^+ (e/z)=597

Anal. Calcd. for $\text{C}_{36}\text{H}_{36}\text{O}_8$ (596, 36): C, 72.50; H, 6.04

Found: C, 72.67; H, 6.26.

5,17-diformyl-26,28-dihydroxy-25,27-bis(ethoxycarbonyl-methoxy)calix[4]arene (2)

2 g (3.4 mmol) 1 and 6.0 g (4.6 mL; 52 mmol) α,α -dichloromethylmethylether are dissolved in 100 mL CHCl_3 .
5 20 g (11.6 mL; 105 mmol) TiCl_4 is added slowly from a dripping funnel while keeping the temperature below 30°C . The solution turns dark red and after 30-45 minutes (followed by thin layer chromatography) at room temperature, the reaction mixture is quenched with 5%
10 HCl /ice and extracted with 2 x 50 mL CH_2Cl_2 . The organic phase is purple, probably due to formation of titanium complexes which can be decomposed by several extractions with semi-concentrated HCl . The organic phase is dried with MgSO_4 , filtered, and the solvent is evaporated to give a yellowish compound.

Yield: 2.5 g (100%).

Melting point: $[180-182]^\circ\text{C}$

^1H NMR (CDCl_3) (100 MHz) 1.35(t, 6H, $J=7.20$ Hz), 3.50
(d, 4H, $J=13.0$ Hz), 4.35(q, 4H, $J=7.20$ Hz), 4.45(d, 4H,
20 $J=13.0$ Hz), 4.71(s, 4H), 6.75-7.25(m, 6H), 7.61(s, 4H),
8.70(s, 2H), 9.77(s, 2H)

IR (KBr): 1682 cm^{-1} (s, C=O formyl), 1752 cm^{-1} (s, C=O ester), 3364 cm^{-1} (b, -OH)

M^+ (m/e)=653

25 Anal. Calcd. for $\text{C}_{38}\text{H}_{38}\text{O}_{10}$ (654.38): C, 69.74; H, 5.81
Found: C, 65.03; H, 5.50 (+ an uncombusted rest!).

Diesterdiquinone (3)

2.1 g (4.7 mmol) $\text{Ti}(\text{NO}_3)_3 \cdot 3\text{H}_2\text{O}$ is placed in a 500 mL
30 flask under N_2 and dissolved in a mixture of 150 mL absolute EtOH and 100 mL dry MeOH. A solution of 0.50 g (0.84 mmol) 1 in 50 mL CHCl_3 is added quickly. The solution turns yellow immediately and after 2-3 minutes a precipitate is formed. Upon standing for 15-30 minutes
35 with stirring followed by quenching with 20 mL H_2O , 10%

HCl is added dropwise until the precipitate is dissolved. The reaction mixture is transferred to a separation funnel together with 100 mL CHCl₃ and 50 mL H₂O. The organic phase is isolated and dried with MgSO₄ and the solvent is evaporated. Purification is performed on silica with 2% MeOH in CH₂Cl₂ as eluent, and the yellow band with a R_f=0.45 is collected.

Yield: 0.340 g (66%).

Melting point [203-206]°C

¹³C NMR (CDCl₃) 13.54, 29.84, 62.07, 70.64, 70.78, 124.93, 129.32, 129.77, 132.87, 147.38, 170.39, 186.76, 187.76

¹H NMR (CDCl₃) δ (250 MHz) 1.21(t, 6H, J=7.1 Hz), 3.05 (d, 4H, J=12.9 Hz), 3.88(d, 4H, J=12.9 Hz), 4.02(s, 4H), 4.25(q, 4H, J=7.1 Hz), 6.61(s, 4H), 6.66(s, 4H)

IR (KBr): 1677 cm⁻¹(s, C=O quinone), 1738 cm⁻¹(s, C=O ester)

M⁺(m/e)=625

Anal. Calcd. for C₃₆H₃₂O₁₀TlCl (864.18): C, 50.03; H, 3.70

Found: C, 47.21; H, 3.56 (+ an uncombusted rest!).

5,17-bis(4-nitrophenyldiazo)-26,28-dihydroxy-25,27-bis(ethoxycarbonylmethoxy)calix[4]arene (4)

0.50 g (0.84 mmol) 1 is dissolved with stirring in 50 mL THF and 3 mL pyridine. The reaction mixture is cooled on ice. 0.58 g (2.45 mmol) 4-nitrophenyldiazonium tetrafluoroborate is added in small portions to ensure that the temperature does not exceed 5°C. After stirring and cooling for 2 hours the temperature is allowed to rise to room temperature and the reaction is left for another 14 hours. The solvent is evaporated and the red solid is dissolved in 50 mL CH₂Cl₂ and extracted with 2 x 50 mL 5% HCl. The organic phase is dried with MgSO₄, and the solvent is evaporated to give a red semi-solid. The solid is purified on a short

silica column with CH_2Cl_2 as eluent and isolated as a foam after removing the solvent. The foam is dissolved in a small amount of CH_2Cl_2 and precipitated with EtOH, filtered and washed with EtOH. The resulting substance is airdried.

Yield: 0.30 g (40%).

Melting point [256-258]°C

^{13}C NMR (CDCl_3) 14.06, 31.33, 61.53, 72.38, 122.76, 124.59, 124.62, 125.87, 128.65, 129.55, 132.27, 145.67, 147.87, 152.13, 156.24, 157.84, 168.70

^1H NMR (CDCl_3) δ (250 MHz) 1.37(t, 6H, J=7.2 Hz), 3.56 (d, 4H, J=13.3 Hz), 4.37(q, 4H, J=7.2 Hz), 4.52(d, 4H, J=13.3 Hz), 4.76(s, 4H), 6.81(t, 2H, J=7.5 Hz), 7.03(d, 4H, J=7.5 Hz), 7.79(s, 4H), 7.94(d, 4H, J=9.0 Hz), 8.34(d, 4H, J=9.0 Hz), 8.58(s, 2H)

IR (KBr): 1522 cm^{-1} (s, $-\text{NO}_2$), 1751 cm^{-1} (s, C=O ester), 3392 cm^{-1} (b, -OH)

$\text{M}^+(\text{m/e})=895$

Anal. Calcd. for $\text{C}_{48}\text{H}_{42}\text{N}_6\text{O}_{12}$ (894.48): C, 64.45; H, 4.70;

N, 9.39

Found: C, 62.32; H, 4.76; N, 8.66.

5,17-bis(2,4-dinitrophenyldiazo)-26,28-dihydroxy-25,27-bis(ethoxycarbonylmethoxy)calix[4]arene (5)

0.67 g (1.1 mmol) 3 is dissolved in a mixture of 20 mL CHCl_3 and 20 mL MeOH. 1.0 g (2.5 mmol) 2,4-dinitrophenylhydrazine (50% in H_2O) is dissolved in about 80 mL MeOH/ CHCl_3 and added with stirring to the solution of 3. Then the reaction mixture is heated at reflux for 2 hours and left for 14 hours at room temperature. The solution is filtered to give red crystals. The crystals are dissolved in a small amount of CHCl_3 and triturated with MeOH to give glistening crystals.

Yield: 0.65 g (61%).

Melting point [254-256]°C

- ^{13}C NMR (CDCl_3) 12.73, 29.73, 60.19, 71.13, 118.77, 119.09, 124.24, 124.85, 126.64, 127.69, 128.29, 131.16, 144.58, 145.52, 147.67, 150.93, 157.61, 167.27
- ^1H NMR (CDCl_3) δ (250 MHz) 1.38(t, 6H, J=7.2 Hz),
5 3.56(d, 4H, J=13.3 Hz), 4.37(q, 4H, J=7.2 Hz),
4.52(d, 4H, 13.3 Hz), 4.76(s, 4H), 6.81(t, 2H, J=7.5 Hz),
7.03(d, 4H, J=7.5 Hz), 8.45(s, 4H), 8.49(d, 4H, J=9.0 Hz),
8.76(d, 4H, J=9.0 Hz), 8.84(s, 2H)
- IR (KBr): 1346 cm^{-1} og 1535 cm^{-1} (s, $-\text{NO}_2$), 1747 cm^{-1} (s, C=O
10 ester), 3401 cm^{-1} (b, $-\text{OH}$)
- M^+ (m/e)=985
- Anal. Calcd. for $\text{C}_{48}\text{H}_{40}\text{N}_8\text{O}_{16}$ (984.48): C, 58.56; H, 4.06;
N, 11.38
- Found: C, 57.69; H, 3.85; N, 11.12.
- 15
- 5,17-bis(2,4-dinitrophenyldiazo)-26,28-dihydroxy-25,27-bis(hydroxycarbonylmethoxy)calix[4]arene (6)
- 0.10 g (0.1 mmol) 5 is dissolved in 20 mL EtOH and 10 mL H_2O and then heated to reflux. 0.07 g (0.6 mmol)
- 20 potassium tert.butoxide is added and the reaction is refluxed for 30 minutes. After cooling to room temperature 30 mL 5% HCl is added, and the reaction mixture is then cooled to 5°C. The red precipitate is collected by
- 25 centrifugation and washed twice with H_2O . The precipitate is transferred to a roundbottom flask with EtOH and the solvent is removed to give a red powder.
- Yield: 0.091 g (97%).
- Melting point > 345°C
- IR (KBr): 1345 cm^{-1} og 1510 cm^{-1} (s, $-\text{NO}_2$), 1730 cm^{-1} (s, C=O
30 acid), 3425 cm^{-1} (b, $-\text{OH}$)
- Anal. Calcd. for $\text{C}_{44}\text{H}_{32}\text{N}_8\text{O}_{16}$ (928.44): C, 56.92; H, 3.45;
N, 12.06
- Found: C, 54.73; H, 3.49; N, 11.40.

5,17-bis(1-dicyanovinylinden-3-one)-26,28-dihydroxy-
25,27-bis(ethoxycarbonylmethoxy)calix[4]arene (7)

0.36 g (0.55 mmol) 2 and 0.25 g (1.3 mmol) 1-dicyanovinylinden-3-one are dissolved in 20 mL absolute EtOH
5 with heating. The solution turns red and after 2 hours at reflux the reaction mixture is allowed to cool to room temperature, then the precipitate is filtered off and washed with EtOH.

Yield: 0.45 g (80%).

10 Melting point [286-289]°C

¹³C NMR (CDCl₃) 14.05, 31.10, 61.60, 72.39, 114.35, 114.57, 123.82, 124.80, 124.95, 125.63, 126.12, 126.29, 128.54, 129.56, 129.76, 130.86, 132.03, 134.39, 134.89, 136.99, 137.28, 139.44, 148.183, 151.80, 160.16,

15 162.83, 168.53, 190.72

¹H NMR (CDCl₃) δ (250 MHz) 1.38(t, 6H, J=7.1 Hz), 3.57(d, 4H, J=13.4 Hz), 4.38(q, 4H, J=7.1 Hz), 4.46 (d, 4H, J=13.4 Hz), 4.75(s, 4H), 6.89(t, 2H, J=7.7 Hz), 7.10(d, 4H, J=7.7 Hz), 7.23(t, 2H, J₀=7.2 Hz, J_m=1.28 Hz),
20 7.76(t, 2H, J₀=7.2 Hz, J_m=1.28 Hz), 7.93(d, 2H, J₀=7.2 Hz, J_m=1.28 Hz), 8.24(s, 4H), 8.67(d, 2H, J₀=7.2 Hz, J_m=1.28 Hz), 9.10(s, 2H)

IR (KBr): 1704 cm⁻¹(s, C=O inden), 1747 cm⁻¹(s, C=O ester), 2221 cm⁻¹(m, CN), 3387 cm⁻¹(b, -OH)

25 M⁺(m/e)=1005

Anal. Calcd. for C₆₂H₄₄N₄O₁₀(1004.62): C, 74.12; H, 4.38; N, 5.57

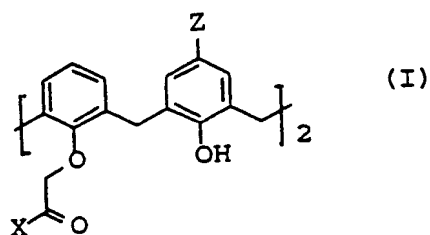
Found: C, 72.29; H, 4.48; N, 4.88.

C L A I M S

1. A calix[4]arene compound,
characterized by the general formula

5

10



wherein

15

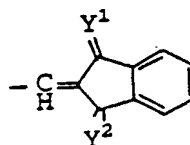
X is -OH, -OR¹, -NR², or morpholino,

whereby R¹ is straight chain or branched alkyl of 1-22 C-atoms and R² is straight chain or branched alkyl of 1-12 C-atoms, and

20

Z is -N=N-Ar, -CH=CH-Ar, -CH=CZ¹Z² or

25



30

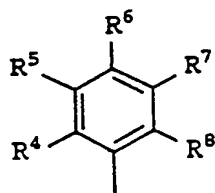
whereby either of Z¹ and Z² are selected from
-H, -NO₂, -CN, -CF₃, -SOR³, -SO₂R³, -SO₂OR³,
-SO₂NHR³, -SO₃H, -COOR³, -COONR³, -COONHR³,
-COOH, -CHO, -COR³, -F, -Cl and -Br, R³ is
straight chain or branched alkyl of 1-4 C-
atoms, and both of Z¹ and Z² are not -H;

35

either of Y^1 and Y^2 are selected from $=O$,
 $=N-CN$ and $=C(CN)_2$; and

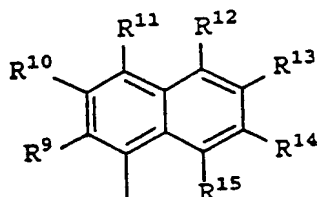
Ar is

5

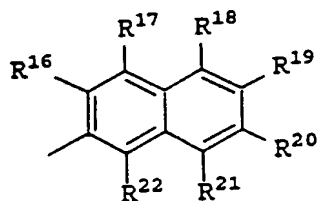


(Substituted phenyl),

10

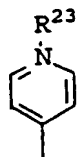


(Substituted 1-naphthyl),



(Substituted 2-naphthyl) or

15



(4-Pyridylium),

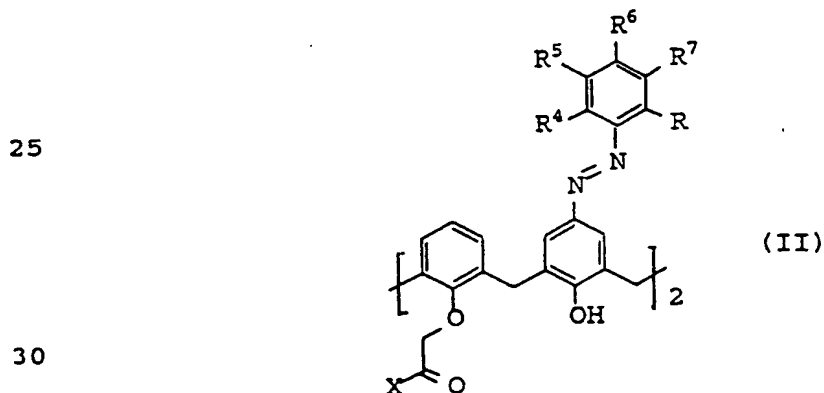
20

and R^4, R^5, \dots, R^{22} are each selected from $-H$,
 $-NO_2$, $-CN$, $-CF_3$, $-F$, $-Cl$, $-Br$, $-SOR^{24}$, $-SO_2R^{24}$,
 $-SO_2CH_2CH_2OR^{25}$, $-SO_2OR^{24}$, $-SO_2NHR^{24}$, $-SO_3H$, $-COOR^{24}$,
 $-CONR^{24}$, $-CONHR^{24}$, $-COOH$, $-CHO$ and $-COR^{24}$,
 wherein R^{24} is straight chain or branched al-

kyl of 1-4 C-atoms, and R^{25} is -H, $-SO_3H$,
 $-SO_3Li$, $-SO_3Na$ or $-SO_3K$,

5 with the proviso that when Z is $-CH=CHAR$ and
 Ar is phenyl, at least one of the substitu-
 ents $R^4, R^5, \dots R^8$ of the phenyl group must be
 different from H, and when Z is $-CH=CHAR$ and
 Ar is 1-naphthyl, at least one of the sub-
 10 stituents $R^9, R^{10}, \dots R^{15}$ of the 1-naphthyl group
 must be different from H, and when Z is
 $-CH=CHAR$ and Ar is 2-naphthyl, at least one of
 the substituents $R^{16}, R^{17}, \dots R^{22}$ of the 2-naphthyl
 group must be different from H.

- 15 2. A calix[4]arene compound according to claim 1,
 wherein Ar is a phenyl group having at least one
 sulphoxylate substituent.
- 20 3. A calix[4]arene compound according to claim 2,
 characterized by the general formula



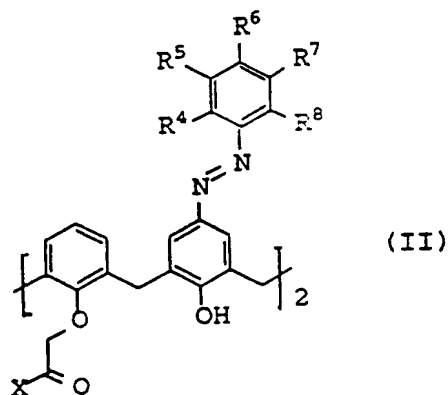
35 wherein $R^4, R^5, \dots R^8$ each are selected from -H and
 $-SO_2CH_2CH_2OR^{25}$; $R^4, R^5, \dots R^8$ not all being -H, R^{25} is

-H, -SO₃H, -SO₃Li, -SO₃Na or -SO₃K, and X is -OH or -OR¹, whereby R¹ is straight chain or branched alkyl of 1-22 C-atoms.

- 5 4. A calix[4]arene compound according to claim 3, wherein R²⁵ is -SO₃H, -SO₃Li, -SO₃Na or -SO₃K.
5. A calix[4]arene compound according to claim 1, wherein Ar is a phenyl group having at least one
10 substituent of the type -NO₂, -CN or Cl.
6. A calix[4]arene compound according to claim 5, characterized by the general formula

15

20



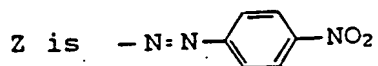
25

wherein R⁴, R⁵, ..., R⁸ each are selected from -H, -NO₂, -CN and -Cl; R⁴, R⁵, ..., R⁸ not all being -H, and X is -OH or -OR¹, whereby R¹ is straight chain or
30 branched alkyl of 1-22 C-atoms.

30

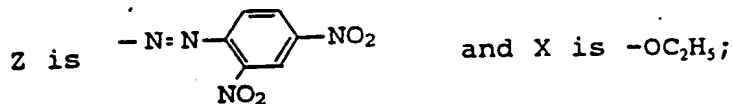
7. A calix[4]arene compound according to claim 1, wherein

35

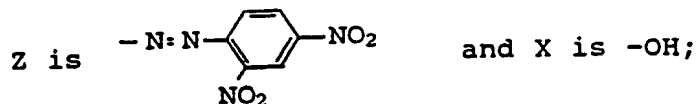


and X is -OC₂H₅;

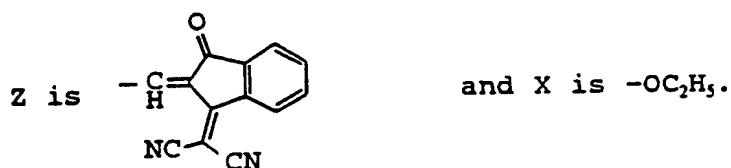
25



5

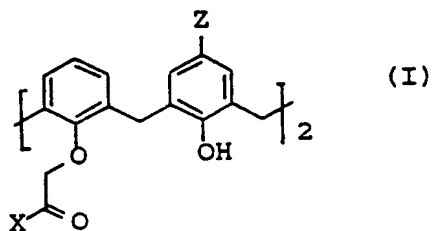


10



8. A calcium sensitive sensor having a calcium sensitive area containing an immobilized calcium sensitive active component,
 wherein the calcium sensitive active component is a compound of the general formula

20



25

wherein

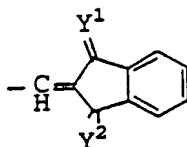
30

X is $-\text{OH}$, $-\text{OR}^1$, $-\text{NR}^2$ or morpholino,

whereby R^1 is straight chain or branched alkyl of 1-22 C-atoms and R^2 is straight chain or branched alkyl of 1-12 C-atoms, and

35

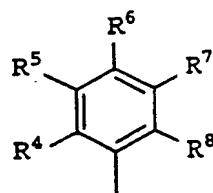
Z is $-N=N-Ar$, $-CH=CH-Ar$, $-CH=CZ^1Z^2$ or



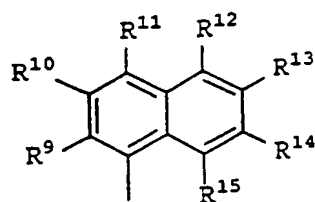
10
 whereby either of Z^1 and Z^2 are selected from
 $-H$, $-NO_2$, $-CN$, $-CF_3$, $-SOR^3$, $-SO_2R^3$, $-SO_2OR^3$,
 $-SO_2NHR^3$, $-SO_3H$, $-COOR^3$, $-COONR^3_2$, $-COONHR^3$,
 $-COOH$, $-CHO$, $-COR^3$, $-F$, $-Cl$ and $-Br$, R^3 is
 straight chain or branched alkyl of 1-4 C-
 atoms and both of Z^1 and Z^2 are not $-H$;

15
 either of Y^1 and Y^2 are selected from $=O$,
 $=N-CN$ and $=C(CN)_2$; and

Ar is

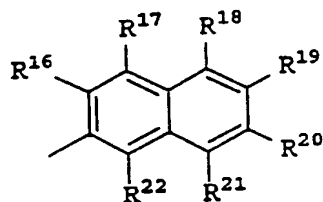


(Substituted phenyl),

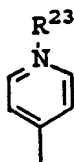


(Substituted 1-naphtyl),

27



(Substituted 2-naphtyl) or



(4-Pyridylum),

5

and R^4, R^5, \dots, R^{22} are each selected from -H, -NO₂, -CN, -CF₃, -F, -Cl, -Br, -SOR²⁴, -SO₂R²⁴, -SO₂CH₂CH₂OR²⁵, -SO₂OR²⁴, -SO₂NHR²⁴, -SO₃H, -COOR²⁴, -CONR²⁴, -CONHR²⁴, -COOH, -CHO and -COR²⁴,
 10 wherein R²⁴ is straight chain or branched alkyl of 1-4 C-atoms, and R²⁵ is -H, -SO₃H, -SO₃Li, -SO₃Na or -SO₃K,

15

20

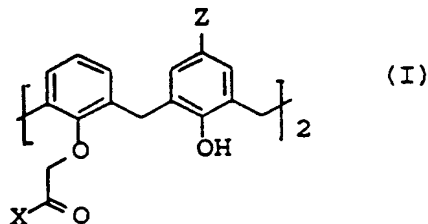
with the proviso that when Z is -CH=CHAr and Ar is phenyl, at least one of the substituents R^4, R^5, \dots, R^8 of the phenyl group must be different from H, and when Z is -CH=CHAr and Ar is 1-naphthyl, at least one of the substituents $R^9, R^{10}, \dots, R^{15}$ of the 1-naphtyl group must be different from H, and when Z is -CH=CHAr and Ar is 2-naphtyl, at least one of the substituents $R^{16}, R^{17}, \dots, R^{22}$ of the 2-naphtyl group must be different from H.

25

9. A calcium sensitive sensor according to claim 8, wherein the calcium sensitive active component is immobilized in a polymeric membrane, preferably a hydrophilic polymeric membrane.

10. A calcium sensitive sensor according to claim 8,
wherein the hydrophilic polymeric membrane consists of celluloseacetate, cellophane, cuprophane, polyvinylacetate, polyhydroxyethylmethacrylate or
5 another hydrogel.
11. A calcium sensitive sensor according to claim 8,
wherein a calcium permeable membrane constitutes
an outer surface of the calcium sensitive area,
10 and the calcium sensitive active component is located in a compartment of the sensor adjacent the membrane.
12. A calcium sensitive membrane for a calcium sensitive
15 sensor comprising a matrix and an immobilized calcium sensitive active component in the matrix,
wherein the calcium sensitive active component is a compound of the general formula

20



25

wherein

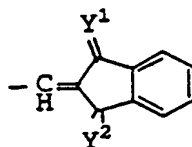
30

X is -OH, -OR¹, -NR², or morpholino,

whereby R¹ is straight chain or branched alkyl of 1-22 C-atoms and R² is straight chain or branched alkyl of 1-12 C-atoms, and

35

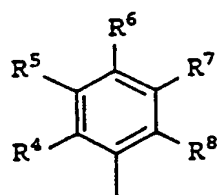
Z is $-N=N-Ar$, $-CH=CH-Ar$, $-CH=CZ^1Z^2$ or



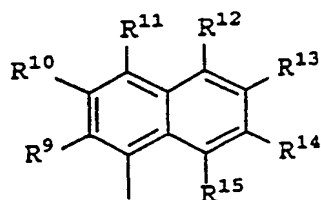
10
 whereby either of Z^1 and Z^2 are selected from
 $-H$, $-NO_2$, $-CN$, $-CF_3$, $-SOR^3$, $-SO_2R^3$, $-SO_2OR^3$,
 $-SO_2NHR^3$, $-SO_3H$, $-COOR^3$, $-COONR^3$, $-COONHR^3$,
 $-COOH$, $-CHO$, $-COR^3$, $-F$, $-Cl$ and $-Br$, R^3 is
 straight chain or branched alkyl of 1-4 C-
 atoms, and both of Z^1 and Z^2 are not $-H$;

15
 either of Y^1 and Y^2 are selected from $=O$,
 $=N-CN$ and $=C(CN)_2$; and

20
 Ar is

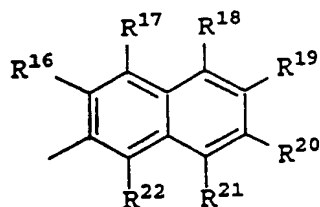


(Substituted phenyl),

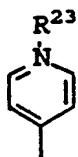


(Substituted 1-naphthyl),

30



(Substituted 2-naphthyl) or



(4-Pyridylum),

5

and R^4, R^5, \dots, R^{22} are each selected from -H, -NO₂, -CN, -CF₃, -F, -Cl, -Br, -SOR²⁴, -SO₂R²⁴, -SO₂CH₂CH₂OR²⁵, -SO₂OR²⁴, -SO₂NHR²⁴, -SO₃H, -COOR²⁴, -CONR²⁴, -CONHR²⁴, -COOH, -CHO and -COR²⁴,
 10 wherein R²⁴ is straight chain or branched alkyl of 1-4 C-atoms, and R²⁵ is -H, -SO₃H, -SO₃Li, -SO₃Na or -SO₃K,

15

20

with the proviso that when Z is -CH=CHAr and Ar is phenyl, at least one of the substituents R^4, R^5, \dots, R^8 of the phenyl group must be different from H, and when Z is -CH=CHAr and Ar is 1-naphthyl, at least one of the substituents $R^9, R^{10}, \dots, R^{15}$ of the 1-naphthyl group must be different from H, and when Z is -CH=CHAr and Ar is 2-naphthyl, at least one of the substituents $R^{16}, R^{17}, \dots, R^{22}$ of the 2-naphthyl group must be different from H.

25

13. A calcium sensitive membrane according to claim 12,
 wherein the matrix consists of a polymeric material, preferably a hydrophilic polymeric material

such as celluloseacetate, cellophane, cuprophane, polyvinylacetate, polyhydroxyethylmethacrylate or another hydrogel.

1/2

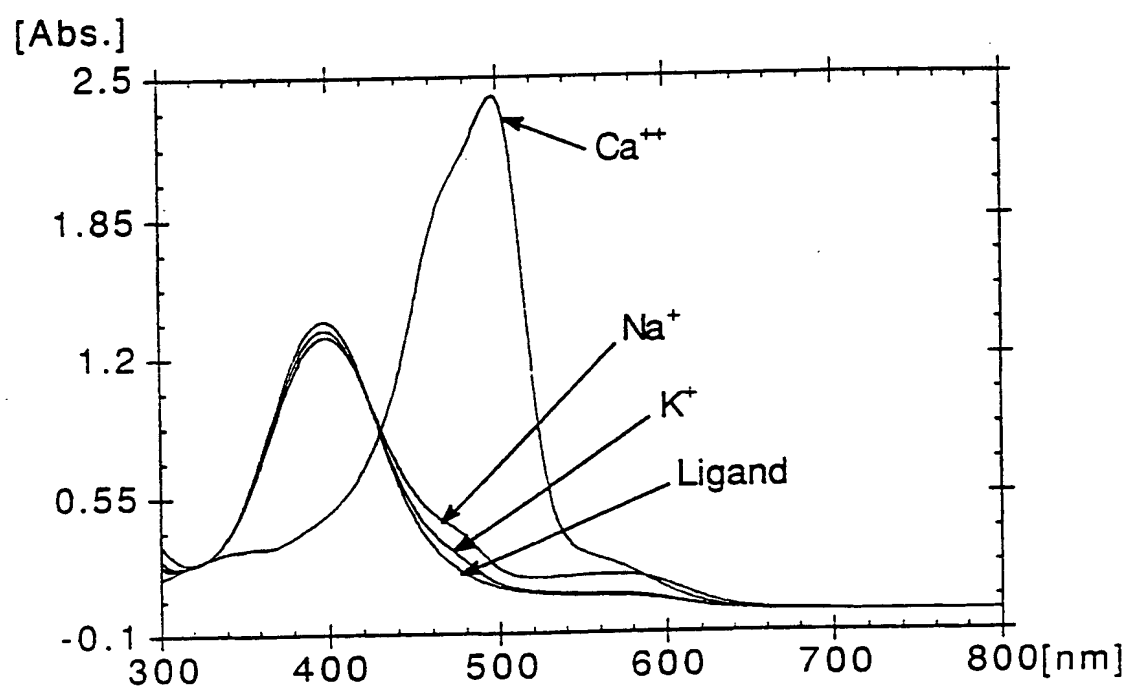


FIG. 1

2/2

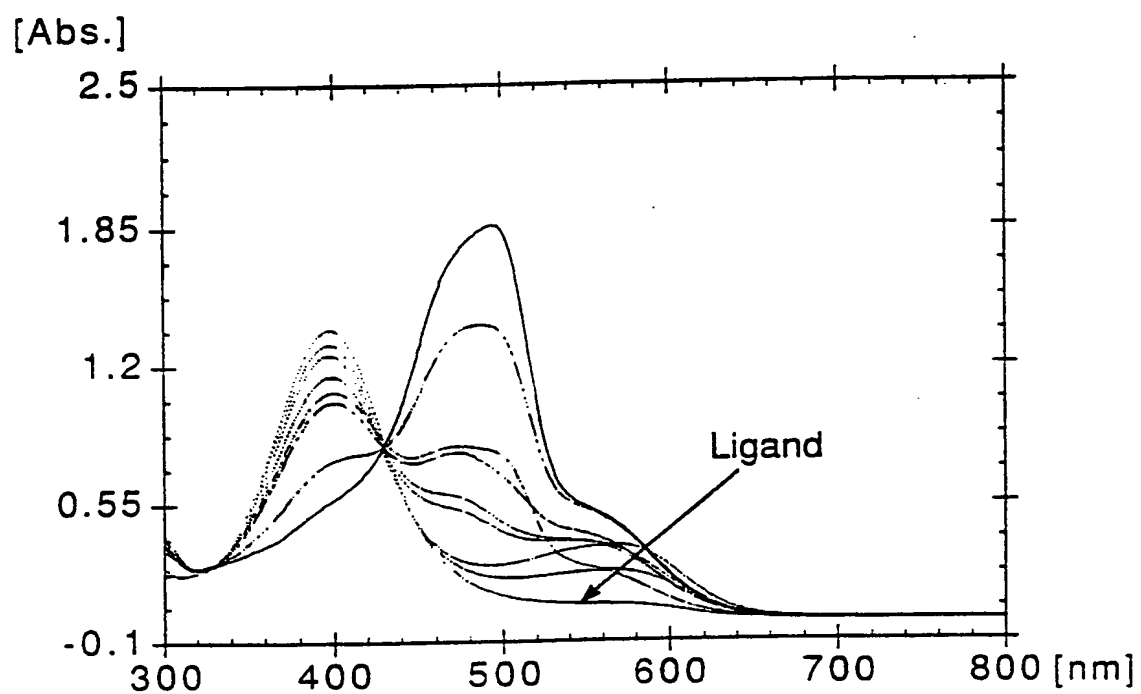


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 94/00254

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5: C07C 245/08, C07C 255/40, G01N 33/84
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5: C07C, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEM. SOC. CHEM. COMMUN., 1993, Yuji Kubo et al: "Synthesis of a 1,3-Bis(indoaniline)-derived Calix /4/arene as an Optical Sensor for Calcium Ion", see page 305 - page 307 -----	1-13

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

17 October 1994

20 -10- 1994

Name and mailing address of the ISA/

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